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Shelf-Life and Safety Enhancement of Processed Meat by Hydrostatic Pressure in Combination with Moderate Temperature and Biopreservatives, Phase IV

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The effectiveness of a moderate hydrostatic pressure in combination with moderate temperature and biopreservatives to reduce high populations of pathogenic and spoilage bacteria in processed meat products was determined. Roast beef and fermented summer sausage were inoculated with 10(3) to 10(4) cells/g of four pathogens and four spoilage bacteria along with pediocin AcH or one of two bacteriocin-based biopreservatives. After vacuum-packaging, the bags were pretempered in a water bath to 50 C and then pressurized at 50,000 psi for 5 min at 50 C. The products were then stored at 25 C and examined at selected intervals for the presence and numbers of pathogenic and spoilage bacteria during storage up to 12 weeks. The results showed the complete kill of the spoilage and pathogenic bacteria in roast beef as no growth was observed after 12 weeks at 25 C in these samples. Pressurization of processed meat products at 50,000 psi for 5 min at 50 C indicates safety and long shelf-life even during extended storage at abusive temperatures.

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FOOD PRESERVATION, HYDROSTATIC PRESSURE, SHELF LIFE
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PREFACE

This study was conducted from October 1996 through September 1997 by Mssrs. Norasak Kalchayanand and Bibek Ray, University of Wyoming, under the supervision of Drs. Anthony Sikes and Patrick Dunne, Sustainability Directorate, U. S. Army Soldier Systems Command, Natick, MA. The work was funded under the project (DJ10) titled "Antimicrobial effectiveness of ultra-high hydrostatic pressure and pulse electric field in combination with bacteriocins for use in food-preservation", DJ10: C-DAAK60-93-K-0003.

Mssrs. Kalchayanand's and Ray's research was designed to ascertain answers to the following questions: (1) do ultra-hydrostatic pressure (UHP) or electroporation (EP) treatments to pathogenic and spoilage gram-positive and gram-negative bacterial cells induce sublethal injury; (2) do these sublethal injured cells develop susceptibility to antimicrobial peptide of bacteriocins; (3) do UHP or EP treatments in combination with bacteriocins increase greater viability loss of pathogens and spoilage bacteria, and (4) do UHP or EP treatments in combination with lysozyme and bacteriocin enhance viability loss of bacteria.

The research, which was divided into four phases, was initiated on 1 Oct 93. This report summarizes results from phase IV, which ended 30 Sept 97.

SHELF-LIFE AND SAFETY ENHANCEMENT OF PROCESSED MEAT BY HYDROSTATIC PRESSURE IN COMBINATION WITH MODERATE TEMPERATURE AND BIOPRESERVATIVES

PHASE IV

Introduction

Although preservation techniques are used widely, microbial spoilage and health hazards of foods are increasing worldwide. Current consumer demands for foods that appear more natural and fresh have resulted in the search for less drastic food preservation methods. Among several nonthermal processing methods that are being studied, hydrostatic pressure has generated a great deal of interest.

Unlike thermal processing, hydrostatic pressure processing retains physical appearances, color, flavor and nutrients of foods (9 and 12). Hydrostatic pressure is also effective in microbial inactivation at low to moderate temperature and thus can be used to enhance shelf-life and safety of food (10).

Several countries have marketed hydrostatic-pressure-processed foods including Japan (fruit-based products), France (orange juice) and the U.S.A. (avocado spread; 10). The sensitivity to hydrostatic pressure of various microorganisms naturally present or introduced into fresh or processed meat has been reported (4, 20, 21 and 26). Higher temperatures enhance the effects of hydrostatic pressure. Hydrostatic pressure (60,000 psi) treatment of fatty goose or duck liver at 50°C for 10 or 30 min reduced the microbial load as efficiently as the usual thermal pasteurization (about 85°C) without melting or separating lipids (11). The benefits of combined hydrostatic pressure and moderate temperature in

terms of safety, storage life and quality could be extended to many high-valued and heat-sensitive meat products and meat-based ready-to-eat meals (10).

Bacteriocins of lactic acid bacteria are extracellularly released peptide or protein molecules that are bactericidal to gram-positive bacteria, as well as to sublethally injured gram-positive and gram-negative bacteria (16, 24 and 25). Bacteriocins produced by lactic acid bacteria may also be considered as natural preservatives or biopreservatives, which have had increased interest during recent years (27). An increased lethality of pressure to foodborne bacterial cells in the presence of bacteriocins (17) and lysozyme, nisin, and/or EDTA (14 and 17) has been reported. However, results of the combination effect of hydrostatic pressure, moderate temperature and bacteriocin-based biopreservatives on controlling or inactivating microorganisms in processed meat products is limited.

The objectives of these studies were to determine: (a) the effectiveness of hydrostatic pressure in combination with moderate temperature and biopreservatives to reduce high population of spoilage and pathogenic bacteria in processed meat products and (b) the shelf-life and safety enhancement of processed meat products by hydrostatic pressure and biopreservatives at abused temperature (25°C).

METHODS AND MATERIALS

Beef product preparation

1.) Roast beef

Semimembranosus muscles, USDA Select, were purchased from Sun Land Beef Co. (Tolleson, AZ) and were cooked in a Maurer convection computerized smoke house (Reichenau, West Germany). The smoke house was programmed to cook stepwise to an internal temperature of 71.1°C. Each roast beef was chilled at 4°C overnight and aseptically cut perpendicular to the muscle fiber orientation into approximately 84 g (3 oz) per slice.

2.) Cotto Salami

Cattle, yield grade 2, from U. of Wyoming feed lot were killed on March 12, 1997. Cattle carcasses were aged at 1°C in a humidity controlled cooler for 3 weeks before trimming. Cotto Salami was formulated as the follows:

1. Beef trim	**80%**
2. Beef fat	**20%**
3. Salt (Sodium chloride; Heller Seasonings & Ingredients, Inc., Bedford Park, IL)	**1.7%**
4. Modern Cure (Heller Seasonings & Ingredients, Inc., Bedford Park, IL)	**0.25%**
5. Sodium erythorbate (Heller Seasonings & Ingredients, Inc., Bedford Park, IL); 550 ppm.	**0.055%**
6. Sodium tripolyphosphate (Heller Seasonings & Ingredients, Inc., Bedford Park, IL)	**0.3%**

7. Fine ground black pepper (Heller Seasonings & Ingredients, Inc., Bedford Park, IL) 0.3%

8. Garlic powder (Heller Seasonings & Ingredients, Inc., Bedford Park, IL) 0.1%

9. Onion powder (Heller Seasonings & Ingredients, Inc., Bedford Park, IL) 0.1%

10. Water (20% added and 10% for cook loss) 30%

Beef trim and fat were ground through 1", 3/8" and 1/8" plates, respectively, using Biro grinder (The Biro Mfg., Co., Marblehead, OH). Ground beef was mixed at low speed for one min with salt, two min with all dry ingredients and water, and mixed at high speed for one min using a mixer (Berkel BA-20, Berkel Inc., La Porte, IN). When necessary, bacteriocins were added to a final concentration of 3,000 activity units (AU)/g and mixed to form sausage. Raw sausage was stuffed in fiber cellulose casings (Brechteen Co., Chesterfield, MI) using a manual stuffer (F. Dick, Koch, KS). Sausages were weighed before and after cooking to determine the cook yield. Sausages were cooked in a Maurer convection computerized smoke house (Reichenau, West German). The smoke house was programmed to cook stepwise to an internal temperature of 68.3°C (155°F) and showered until the internal temperature reached 32.2°C (90°F). Sausages were chilled at 4°C overnight to equilibrate temperature. Sausages were weighed, vacuum packed, and pasteurized by dipping into a water bath for 2 min at 71.1°C (160°F), and into an ice water bath for 2 min. Pasteurized sausages were stored at 2°C before using.

2.) Sweet and sour summer sausage

a.) Preparation of starter culture

***Pediococcus acidilactici* LB42-932, a producer for pediocin AcH, was grown in Trypticase glucose yeast extract (TGE) broth for 10 h at 37°C. Cells were harvested by centrifugation (Beckman, Fullerton, CA) at 8,000 rpm for 10 min at 4°C, resuspended in 0.1% peptone solution, and incubated at 37°C for one hour before using.**

b.) Sausage preparation

Fed steers, USDA selector choice, were bought from Trues Feed Lot, Laramie, WY and killed on April 3, 1997. Carcasses were aged in a humidity controlled cooler at 1°C for 3 weeks before trimming. Sweet and sour summer sausages were formulated as follows:

1. Beef trim	**80%**
2. Beef fat	**20%**
3. Salt (Sodium chloride; Heller Seasonings & Ingredients, Inc., Bedford Park, IL)	**2.8%**
4. Modern Cure (Heller Seasonings & Ingredients, Inc., Bedford Park, IL)	**0.25%**
5. Sodium erythorbate (Heller Seasonings & Ingredients, Inc., Bedford Park, IL); 550 ppm.	**0.055%**
6. Dextrose (Heller Seasonings & Ingredients, Inc., Bedford Park, IL)	**1.0%**
7. Sucrose (Heller Seasonings & Ingredients, Inc., Bedford Park, IL)	**1.5%**
8. Fine ground black pepper (Heller Seasonings & Ingredients,	

Inc., Bedford Park, IL) 0.2%

9. Coarse ground black pepper (Heller Seasonings & Ingredients, Inc., Bedford Park, IL) 0.2%
10. Garlic powder (Heller Seasonings & Ingredients, Inc., Bedford Park, IL) 0.05%
11. Onion powder (Heller Seasonings & Ingredients, Inc., Bedford Park, IL) 0.05%
12. *Pediococcus acidilactici* LB42-932 starter culture 3.75%

Beef trim and beef fat were ground through 1" and 3/8" plates using a Biro grinder (The Biro Mfg., Co., Marblehead, OH). Ground beef was measured for pH before mixing for one min with salt and modern cure, 1 min with sodium erythorbate, 1 min with dextrose, sucrose and spices, and mixing further for 1 min using a food mixer model 200DA (Leland Detroit Mfg., Co., Detroit, MI). *Pediococcus acidilactici* starter culture was added and mixed for 2 min. Ground beef mixture was then ground through 1/8" plate, in order to distribute all the ingredients through the meat batter, and stuffed into fiber cellulose casings (Brechteen Co., Chesterfield, MI) using an automatic stuffer (Vemag Robot 500, Robert Reiser Co., Inc., Canton, MA). Summer sausages were weighed and incubated at 36.1°C (97°F) in a computerized smoke house (Reichenau, West German) until pH of sausage below 5.0. Summer sausages were then cooked stepwise to an internal temperature of 62.8°C (145°F) and showered until the internal temperature dropped to 32.2°C (90°F). Summer sausages were chilled overnight at 4°C, weighed to determine cook yield, and vacuum packed. Sausages were pasteurized by dipping into a water bath at 71.1°C (160°F)

for 2 min and into an ice bath for 2 min. Summer sausages were stored at 2°C until inoculation.

Bacterial strains and cell preparation

Spoilage bacteria, *Leuconostoc mesenteroides* strain ly and *Lactobacillus sake* FM1 from UW food microbiology stock collection, were grown in deMan Rogosa Sharpe (MRS) broth at 25°C for 16 to 18 h. *Serratia liquefaciens* FM1 and *Pseudomonas fluorescens* FM1 were grown in tryptic soy broth fortified with 0.6% yeast extract (TSBY) at 25°C for 16 to 18 h. Pathogenic bacteria, *Escherichia coli* O157:H7 #932, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* 582 and *Listeria monocytogenes* Scott A, were grown in TSBY at 37°C for 16 to 18 h. Bacterial cells were harvested by centrifugation (Beckman, Fullerton, CA) at 8,000 rpm for 10 min at 4°, washed twice and resuspended in sterile 0.1% peptone solution. The cells were kept in an ice bath before inoculation on sausages.

Biopreservatives preparation

Both bacteriocins and bacteriocin-based biopreservatives were prepared and assayed for activity units as in methods described previously (3 and 32). When necessary, lysozyme hydrochloride (SPA, Bio Spa Division; purified grade) was dissolved in deionized water, membrane filtered and stored at 4°C before using at the final concentration of 100 μg/g of meat product.

Inoculation of bacterial cells on roast beef and summer sausage

Both roast beef and sausages were aseptically cut into approximately 84 g (3.0 oz) slices and pHs were randomly measured in both controls and biopreservatives-treated samples using flat surface combination electrode (Markson Science, Houston, TX). Roast beef or sausage slices, in duplicate, were randomly placed in a plastic bag (Cryovac, Model B-540, Duncan, SC) and spoilage or pathogenic bacterial cells were inoculated on each surface of roast beef at a final concentration of 10^7 to 10^8 cells/g for log-cycle reduction (immediately) study and 10^3 to 10^4 cells/g for shelf-life and safety storage study. When necessary, pediocin AcH (Ped) or bacteriocin based-biopreservatives (BP_X or BP_Y) were added on the surfaces to a final concentration of 3,000 AU/g. All the bags were vacuum-sealed (Webo Matic, Meat Packers & Butchers Supply Co., Los Angeles, CA) and stored at 4°C before pressurization.

Hydrostatic pressurization

The roast beef and sausage samples were placed in a circulating water bath at 53.5°C for 10 min to equilibrate the temperature prior to pressurization. Then the samples were placed in the preheated (50±1°C) fluid (Hydrolubric 2, Houghton International, Valley Forge, PA) in the hydrostatic chamber (10.16 by 25.4 cm; Engineered Pressure Systems, Wilmington, MA). Meat samples were pressurized at 50,000 psi for 5 min and pressurized samples were cooled down rapidly in an ice-bath. Meat samples were stored either at 4°C for log-cycle reduction study or at 25°C up to 12 weeks for storage study. At selected intervals during storage, meat samples were randomly taken for enumeration of colony

forming unit (CFU), for observation of purge for live bacterial cells under phase contrast microscope (Swift Instruments, San Jose, CA) and for observation of color and aroma.

Enumeration for colony forming unit

Twenty-five grams of roast beef or sausage samples were aseptically removed from each bag and placed into a sterile plastic bag (Whirl-Pak, Nasco, Ft. Attkinson, WI). Sterile 0.1% peptone solution, 225 ml, was added. Samples were homogenized for 2 min using a stomacher (Tekmar, Cincinnati, OH). The homogenates were serially diluted and 1 ml was plated in duplicate with specific selective media. MRS agar, adjusted to pH to 5.0 with DL-lactic acid (Sigma Chemicals, Co., St. Louis, MO), was used as a selective medium for *L. mesenteroides* and *L. sake*. Tryptic soy agar, supplemented with 0.6% yeast extract (Difco, Detroit, MI; TSAY) and 2.5% sodium chloride, was used as a selective agar for *S. liquefaciens* and *P. fluorescens*. Violet red bile (VRB; Difco, Detroit, MI), xylose lysine deoxycholate (XLD; Difco, Detroit, MI), TSA with 7.5% sodium chloride, and Modified Oxford agars were used as selective media for *E. coli*, *S. typhimurium*, *S. aureus* and *L. monocytogenes*, respectively. Plates were incubated at 25°C for 2 days for spoilage bacteria and at 37°C for 1 to 2 days for pathogens before counting the CFU.

Activity retention of bacteriocins

For summer sausage, samples of 0.635 cm (0.25") in diameter were taken by coring with a sterile cork borer. Each core sample was placed on plate preseeded with an indicator, *Lactobacillus plantarum* NCDO 955 (3 and 32). Purge from both roast

beef and summer sausage were also taken and 10 μl were tested against the lawn of *L. plantarum* NCDO 955. Plates were incubated at 30°C for 16 to 18 h to observe the clear zone of inhibition.

RESULTS AND DISCUSSION

Cook yield, pH and bacteriocin activity retention

The cook yield of roast beef was not tested. The pH of roast beef was 5.92 and there was no difference in pH among the control and treated roast beef with biopreservatives (Table 1). Purge from treated roast beef showed activity against *L. plantarum* NCDO 955 (Table 1). Both cotto salami and summer sausage were formulated and cook yield, pH and bacteriocins activity retention were determined (Table 1). There was no difference in cook yield for cotto salami formulated with and without bacteriocin-based biopreservatives. The cook yields for cotto salami were 93.9, 93.4 and 93.2 % for control, pediocin formulated, and biopreservatives formulated, respectively (Table 1). The cook yield of sweet & sour summer sausages was 99.7% (Table 1). There was no pH change for cotto salami formulated with or without bacteriocin-based biopreservatives (Table 1). The pHs of cotto salami were 5.99, 5.98 and 5.96 for control, pediocin formulated and biopreservatives formulated, respectively. The pH of summer sausages, however, was 4.95 due to fermentation by *Pediococcus acidilactici* (Table 1). After cooking, both cotto salami and summer sausages, formulated with bacteriocin-based biopreservatives or fermented with *Pediococcus acidilactici*, retained their activity against *Lactobacillus plantarum* NCDO 955 (Table 1).

Table 1. Cook yield, pH and bacteriocin activity retention

Product[a]	Cook yield (%)	pH	Bacteriocin activity[b]
Roast beef			
Control	NT	5.92	-
Ped	NT	5.91	+
BP_X	NT	5.92	+
BP_Y	NT	5.92	+
Cotto salami			
Control	93.9	5.99	-
Ped	93.4	5.98	+
BP	93.2	5.96	+
Summer sausage	99.7	4.95	+

[a] Controls for roast beef and cotto salami had no added bacteriocin. Pediocin AcH (Ped) and biopreservatives (BP) were formulated in cotto salami at the final concentration of 3,000 AU/g. Summer sausage was fermented with *P. acidilactici* Lb42-932. BP composition is proprietary.

[b] Bacteriocin activity of each product, formulated or added, was tested against the lawn of *L. plantarum* NCDO 955.

Effect of hydrostatic pressure in combination with moderate temperature (50°C) and biopreservatives on reduction of high-population of spoilage bacteria inoculated in processed meat products

Pressurization at 50,000 psi for 5 min at 50°C reduced the spoilage bacterial population by 3.0 to 7.4 logs for roast beef (Figure 1.), 4.6 to 8.4 logs for cotto salami (Figure 2), and 4.9 to 8.4 logs for summer sausage (Figure 3). Pressurization at higher temperature (45 to 50°C) has been reported to enhance the destructive effect on vegetative cells (4 and 29).

The degree of sensitivity to pressurization depended on the bacterial strain. ***S. liquefaciens*** **and** ***P. fluorescens*** **were the most sensitive whereas** ***L. sake*** **was the most resistant to pressurization. The degree of inactivation by pressurization also depended on the environment of meat products. The highest inactivation of** ***L. mesenteroides*** **and** ***L. sake*** **occurred in summer sausage followed by cotto salami and roast beef (Figure 1, 2 and 3). This sequence may be due to low pH of summer sausage and pediocin AcH produced in meat during the fermentation process. Some survivors of** ***L. mesenteroides*** **and** ***L. sake*** **were found in summer sausages after pressurization probably because both organisms are acid-producing and aciduric bacteria (31). Hydrostatic pressure of cotto salami, formulated with pediocin AcH and inoculated with** ***L. mesenteroides*****, had less viability loss than roast beef with pediocin. This fact may be due to an interaction between pediocin and tripolyphosphate in cotto salami. Pressurization in the presence of biopreservatives reduced more of the bacterial population compared to pressurization alone in all three products (Figures 1, 2 and 3). There were no survivors except** ***L. sake*** **when inoculated into roast beef, cotto salami**

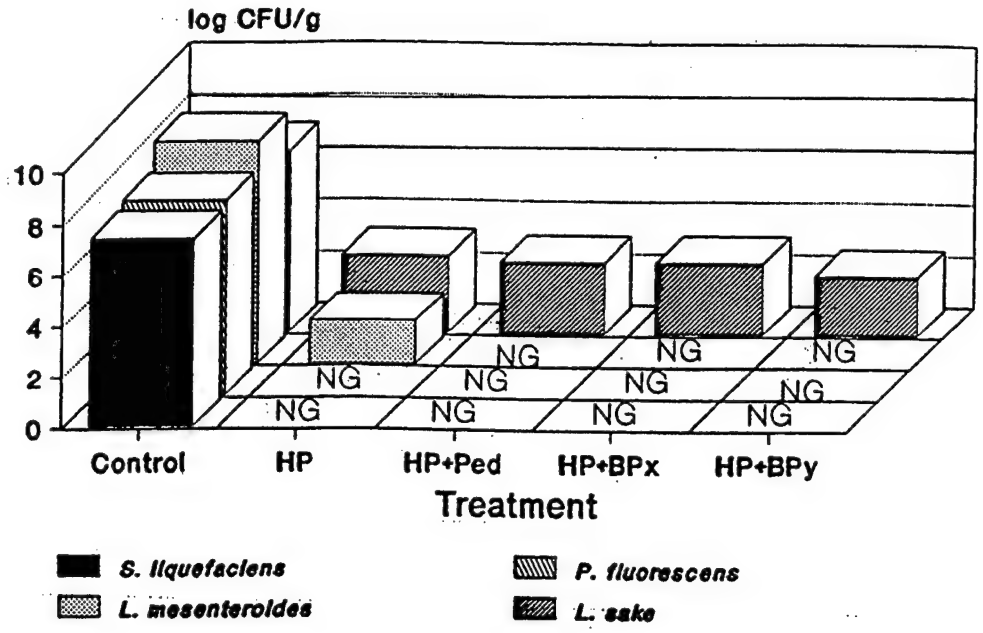

Figure 1. Viability loss of spoilage bacteria inoculated in roast beef subjected to 50,000 psi at 50°C in combination with biopreservatives. Control was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) and biopreservatives (BP_X or BP_Y) were added to the final concentration of 3,000 AU/g. When viable cells were not detected at a level of 0.1g/ml, the cell concentration was reported as *no growth* (NG). BP_X and BP_Y are bacteriocin-based biopreservatives and their compositions are proprietary.

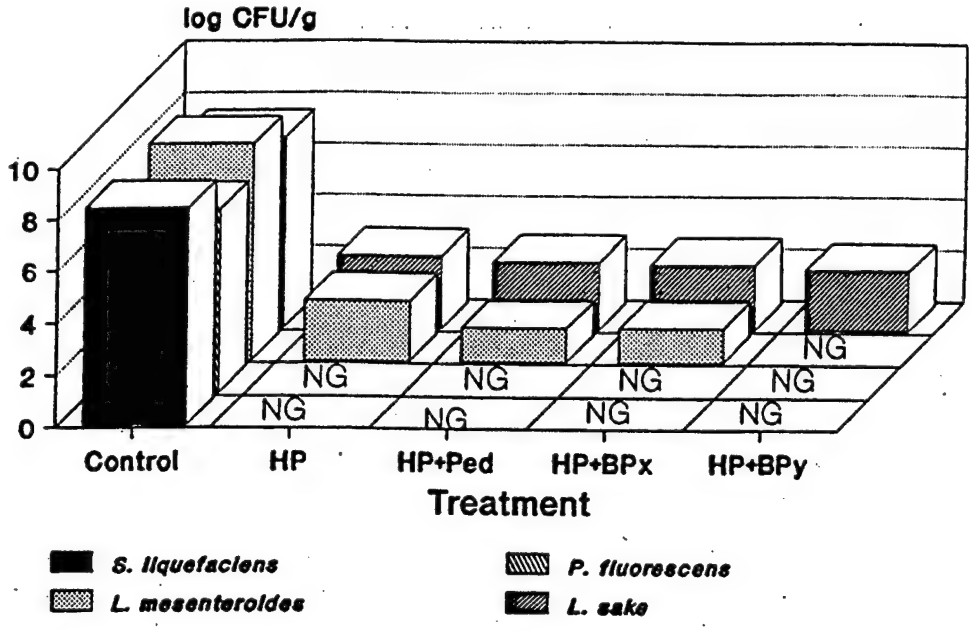

Figure 2. Viability loss of spoilage bacteria inoculated into cotto salami subjected to 50,000 psi at 50°C in combination with biopreservatives. Control was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) and biopreservatives (BP_X or BP_Y) were added to the final concentration of 3,000 AU/g. When viable cells were not detected at a level of 0.1g/ml, the cell concentration was reported as *no growth* (NG). BP_X and BP_Y are bactriocin-based biopreservatives and their compositions are proprietary.

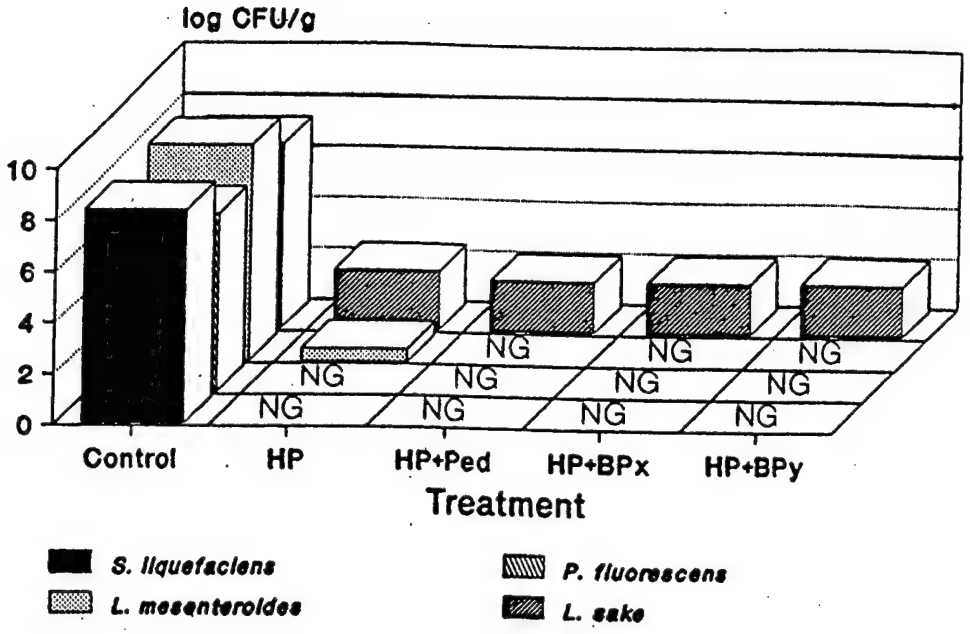

Figure 3. Viability loss of spoilage bacteria inoculated in summer sausage subjected to 50,000 psi at 50°C in combination with biopreservatives. Control was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) and biopreservatives (BP_X or BP_Y) were added to the final concentration of 3,000 AU/g. When viable cells were not detected at a level of 0.1g/ml, the cell concentration was reported as *no growth* (NG). BP_X and BP_Y are bacteriocin-based biopreservatives and their compositions are proprietary.

or summer sausage samples that were pressurized with BP_Y (Figures 1, 2 and 3). This may be due to the differences in sensitivity of bacterial strains to bacteriocins under this environment.

Effect of hydrostatic pressure in combination with moderate temperature (50°C) and biopreservatives on reduction of high population of pathogenic bacteria inoculated in processed meat products

The reduction of high populations of pathogens inoculated on roast beef, cotto salami and summer sausage was presented (Figures 4, 5 and 6). Pressurization at 50,000 psi for 5 min at 50 °C caused some viability loss to all pathogens tested. The viability loss ranged from 4.1 to 7.1 logs in roast beef, from 6 to 7.7 logs in cotto salami, and from 6.6 to 8.1 logs in summer sausage (Figures 4, 5 and 6). On all processed meat products, *S. typhimurium* was the most sensitive to pressurization at 50,000 psi at 50°C and *S. aureus* was the most resistant (Figures 4, 5 and 6). The highest inactivation of pathogens tested occurred in summer sausage due to a combination of pediocin (from fermentation) and low pH. The effect of hydrostatic pressure in conjunction with pH reduction was reported by Mackey et al., (19) and Stewart et al., (29). Pressurization in the presence of either pediocin AcH or biopreservatives caused more viability loss compared to hydrostatic pressure alone, because high pressure sensitizes the outer membrane (> porosity) of bacteria to bioperservatives (14). The viable cells of *E. coli* O157:H7, *S. typhimurium, S. aureus* and *L. monocytogenes* Scott A were not detected (<10 cells/g), when roast beef, cotto salami or summer sausage were pressurized with BP_X (except for *S. aureus* in summer

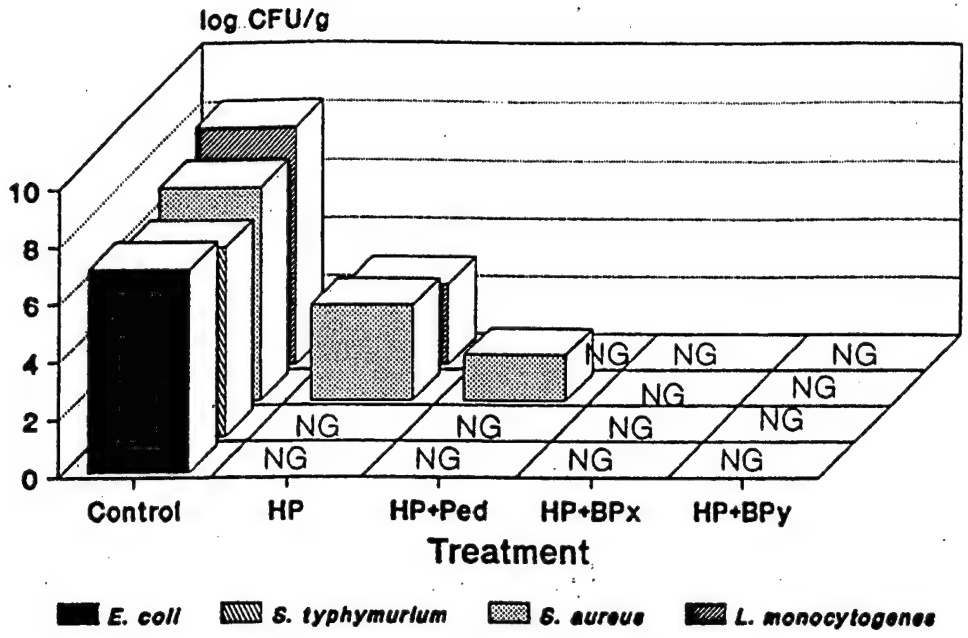

Figure 4. Viability loss of pathogenic bacteria inoculated in roast beef subjected to 50,000 psi at 50°C in combination with biopreservatives. Control was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) and biopreservatives (BP_X or BP_Y) were added to the final concentration of 3,000 AU/g. When viable cells were not detected at a level of 0.1g/ml, the cell concentration was reported as *no growth* (NG). BP_X and BP_Y are bacteriocin-based biopreservatives and their compositions are proprietary.

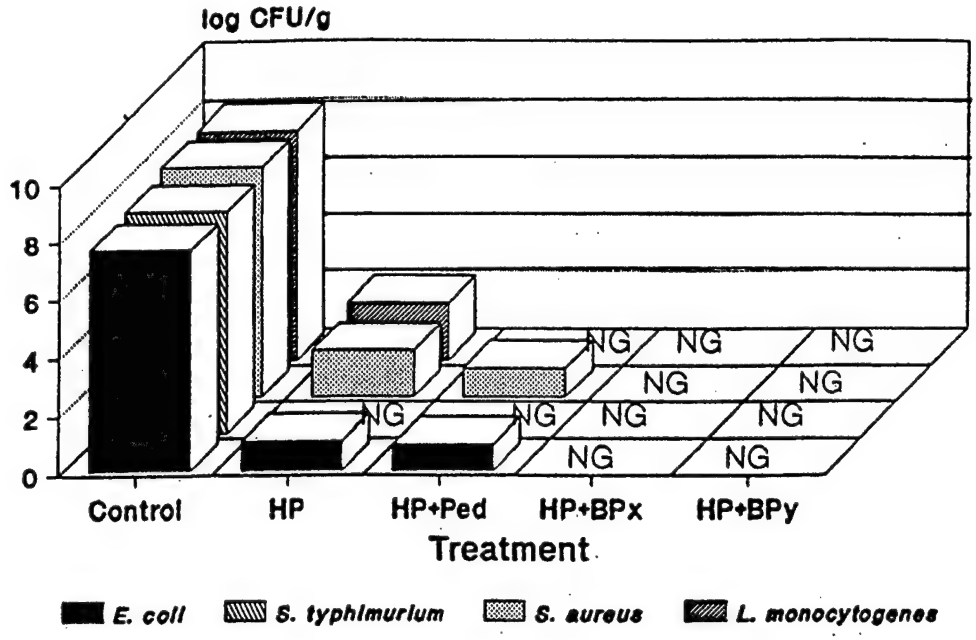

Figure 5. Viability loss of pathogenic bacteria inoculated in cotto salami subjected to 50,000 psi at 50°C in combination with biopreservatives. Control was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) and biopreservatives (BP_X or BP_Y) were added to the final concentration of 3,000 AU/g. When viable cells were not detected at a level of 0.1g/ml, the cell concentration was reported as *no growth* (NG). BP_X and BP_Y are bacteriocin-based biopreservatives and their compositions are proprietary.

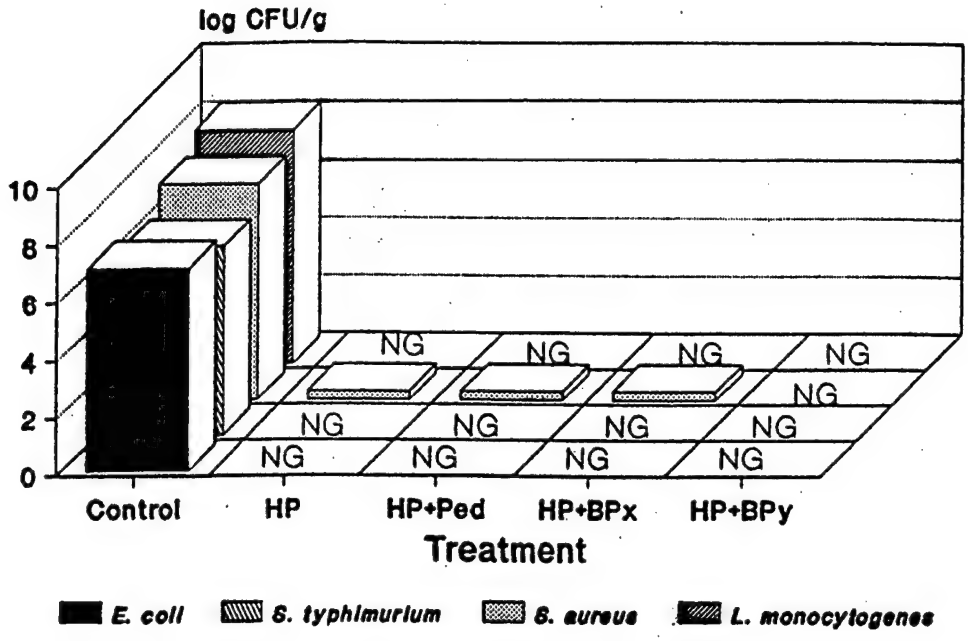

Figure 6. Viability loss of pathogenic bacteria inoculated in summer sausage subjected to 50,000 psi at 50°C in combination with biopreservatives. Control was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) and biopreservatives (BP_X or BP_Y) were added to the final concentration of 3,000 AU/g. When viable cells were not detected at a level of 0.1g/ml, the cell concentration was reported as *no growth* (NG). BP_X and BP_Y are bacteriocin-based biopreservatives and their compositions are proprietary.

sausage) and BP_Y (Figures 4, 5 and 6).

Billions of pounds of domestically produced or imported processed meat products are consumed annually in the U.S. (30). Most of processed meat products are highly perishable. In most cases, processed meat products have been cured with moderate brine concentrations and often subjected to a pasteurization process (13). Additional ingredients, such as spices, sugar, nonfat dry milk, and nonmeat protein, may be included in many of these products (22). As the products are nonsterile, an abusive storage temperature (above 4°C) will increase public health hazard. According to the Centers for Disease Control and Prevention (CDC) summary of the foodborne diseases in the U.S. associated with meat and poultry from 1978 to 1981, 50%, 46.9% and 32.5 % of outbreaks were caused by *E. coli* gastroenteritis, staphylococcal intoxication, and salmonellosis, respectively (5, 6, 7 and 8). We examined the effectiveness of combination treatments of hydrostatic pressure and biopreservatives on shelf-life and safety of processed meat products during storage at 25°C.

In this study, two kinds of processed meat products, roast beef (pH 5.92) and summer sausage (pH 4.95) were used to study shelf-life and safety enhancement during storage at 25°C after subjecting them to hydrostatic pressurization at 50,000 psi for 5 min at 50°C. Low populations (10^2 to 10^4 cells/g) of spoilage and pathogenic bacteria were inoculated to simulate postcontamination of products before packaging.

<u>Effect of hydrostatic pressure in combination with moderate temperature (50°C) and biopreservatives on shelf-life of spoilage bacteria inoculated in processed meat products at 25°C</u>

S. liquefaciens, *P. fluorescens*, *L. mesenteroides*, and *L. sake* grew very well in roast beef stored at 25°C (Table 2). All spoilage bacteria inoculated on roast beef, except for *P. fluorescens*, grew to the population higher than 10^{12} cells/g within one week. Aerobic *P. fluorescens* population increased to $20x10^6$ cells/g due to a micro-aerobic environment (28) after vacuum packaging (15). *S. liquefaciens* grew slower in summer sausage than in roast beef (Table 3). However, growth of *L. mesenteroides* and *L. sake* reached higher than 10^{12} cells/g within a week, indicating that both organisms are acid-tolerant bacteria (31). Growth of *P. fluorescens* on summer sausage gradually declined with increasing storage time, probably due to low acid of the product. The minimum growth pH of most *Pseudomonas* is 5.6 (1). Pressurization at 50,000 psi for 5 min at 50°C with or without biopreservatives killed all spoilage bacteria (Table 2 and 3). There was no growth detected in treated roast beef or summer sausage after storage at 25°C for 12 weeks.

Effect of hydrostatic pressure in combination with moderate temperature (50°C) and biopreservatives on shelf-life of pathogenic bacteria inoculated in processed meat products at 25°C

E. coli O157:H7, *S. typhimurium*, *S. aureus*, and *L. monocytogenes* Scott A grew rapidly in control roast beef at 25°C. The growth of all pathogens tested went up to higher than 10^8 cells/g within a week (Table 4). Unlike roast beef, the growth of pathogens tested in summer sausage decreased with increasing storage time at 25°C (Table 5). The gradual decline in population may not be attributable only to low pH of summer sausage. Generally, the minimum growth pHs for *E. coli*, *S. typhimurium*, *S. aureus*, and

Table 2. Effect of hydrostatic pressure in combination with bacteriocin-based biopreservatives on spoilage bacteria inoculated in roast beef stored at 25°C.

Bacterial strain	Treatment[a]	CFU/g at week 0	1	3	7	12
S. liquefaciens	CTRL	$30x10^2$	$>10x10^{11}$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
P. fluorescens	CTRL	$60x10^2$	$20x10^6$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
L. mesenteroides	CTRL	$30x10^3$	$>10x10^{11}$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
L. sake	CTRL	$40x10^2$	$>10x10^{11}$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10

[a] Control (CTRL) was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) was added at the final concentration for 3,000 AU/g. Bacteriocin based biopreservatives (BP_X or BP_Y) were added at the final concentration of 3,000 AU/ml. When cell concentration was higher than 10^6cells/g, the enumeration was discontinued and reported as not tested (NT). When the bacterial cells were not detected at 1:10 dilution (0.1 g in 1 ml), the cell concentration was reported as less than 10 (<10). BP composition is proprietary.

Table 3. Effect of hydrostatic pressure in combination with bacteriocin-based biopreservatives on spoilage bacteria inoculated in summer sausage stored at 25°C.

Bacterial strain	Treatment[a]	CFU/g at week 0	1	3	7	12
S. liquefaciens	CTRL	$60x10^2$	$11x10^4$	$50x10^5$	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
P. fluorescens	CTRL	$10x10^2$	$30x10^1$	$20x10^1$	$4x10^1$	<10
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
L. mesenteroides	CTRL	$60x10^2$	$>10x10^{11}$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
L. sake	CTRL	$30x10^2$	$>10x10^{11}$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10

[a] Control (CTRL) was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) was added at the final concentration for 3,000 AU/g. Bacteriocin based biopreservatives (BP_X or BP_Y) were added at the final concentration of 3,000 AU/ml. When cell concentration was higher than 10^6 cells/g, the enumeration was discontinued and reported as not tested (NT). When the bacterial cells were not detected at 1:10 dilution (0.1g in1 ml), the cell concentration was reported as less than 10 (<10). BP composition is proprietary.

Table 4. Effect of hydrostatic pressure in combination with bacteriocin-based biopreservatives on pathogenic bacteria inoculated in roast beef stored at 25°C.

Bacterial strain	Treatment[a]	CFU/g at week 0	1	3	7	12
E. coli O157:H7	CTRL	$50x10^2$	$20x10^9$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	P+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
S. typhimurium	CTRL	$15x10^3$	$40x10^9$	NT	NT	NT
	HP	<10	<10	$>10x10^{11}$	NT	NT
	HP+Ped	<10	<10	$>10x10^{11}$	NT	NT
	HP+BP_X	<10	<10	$12x10^9$	NT	NT
	HP+BP_Y	<10	<10	$14x10^8$	NT	NT
S. aureus	CTRL	$30x10^2$	$40x10^7$	NT	NT	NT
	HP	<10	$40x10^2$	$80x10^7$	NT	NT
	HP+Ped	<10	$10x10^1$	$14x10^5$	NT	NT
	HP+BP_X	<10	$10x10^1$	$14x10^5$	NT	NT
	HP+BP_Y	<10	$10x10^1$	$80x10^4$	NT	NT
L. monocytogenes	CTRL	$60x10^2$	$13x10^9$	NT	NT	NT
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10

[a] Control (CTRL) was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) was added at the final concentration of 3,000 AU/g. Bacteriocin based biopreservatives (BP_X or BP_Y) were added at the final concentration of 3,000 AU/ml. When cell concentration was higher than 10^6 cells/g, the enumeration was discontinued and reported as not tested (NT). When the bacterial cells were not detected at 1:10 dilution (0.1g in 1 ml), the cell concentration was reported as less than 10 (<10). BP composition is proprietary

Table 5. Effect of hydrostatic pressure in combination with bacteriocin-based biopreservatives on pathogenic bacteria inoculated in summer sausage stored at 25°C.

Bacterial strain	Treatment[a]	CFU/g at week				
		0	1	3	7	12
E. coli O157:H7	CTRL	$90x10^1$	$40x10^1$	$1x10^1$	<10	<10
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
S. typhimurium	CTRL	$70x10^1$	$20x10^1$	<10	<10	<10
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
S. aureus	CTRL	$30x10^2$	$40x10^1$	$20x10^1$	$5x10^1$	<10
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10
L. monocytogenes	CTRL	$40x10^2$	$1x10^1$	$0.2x10^1$	<10	<10
	HP	<10	<10	<10	<10	<10
	HP+Ped	<10	<10	<10	<10	<10
	HP+BP_X	<10	<10	<10	<10	<10
	HP+BP_Y	<10	<10	<10	<10	<10

[a] Control (CTRL) was not subjected to any treatments. Hydrostatic pressure (HP) was at 50,000 psi for 5 min at 50°C. Pediocin AcH (Ped) was added at the final concentration for 3,000 AU/g. Bacteriocin based biopreservatives (BP_X or BP_Y) were added at the final concentration of 3,000 AU/ml. When the bacterial cells were not detected at 1:10 dilution (0.1 g in 1 ml), the cell concentration was reported as less than10 (<10). BP composition is proprietary.

L. monocytogenes Scott A are 4.3, 4.0, 4.0 (1), and 4.7, respectively (23). The combination of low pH and pediocin AcH from fermentation process may have increased viability loss during storage at 25°C due to sensitivity of the stressed cells to bacteriocin (16). The low pH also increases the bactericidal effectiveness of undissociated nitrous acid derived from sodium nitrite in the cure salt (18). There were no viable cells detected for *E. coli* and *L. monocytogenes* from roast beef pressurized with or without biopreservatives after 12 weeks storage at 25°C (Table 4). However, the viable cells of *S. typhimurium* and *S. aureus* in roast beef were detected after 3 and 1 week of storage at 25°C, respectively. The growth of *S. typhimurium* and *S. aureus* probably resulted from improper prewarming, which resulted in a very low number of survivors that repaired themselves and resumed growth or did not come in contact with biopreservatives due to their uneven distribution. This result is being carefully studied now. However, roast beef samples pressurized in the presence of biopreservatives had 2 to 3 log cycles lower population than roast beef samples that were pressurized alone (Table 4). No growth was detected from either hydrostatic pressure or hydrostatic pressure in combination with biopreservatives of summer sausage inoculated with *E. coli* O157:H7, *S. typhimurium*, *S. aureus*, or *L. monocytogenes* after 12 weeks of storage at 25°C (Table 5).

During this storage study, a strain of *Clostridium* was isolated from some roast beef samples. The total percentage of clostridial strain, found in some roast beef samples, was 3.9%. The percentages found in each treatment sample were 1.6, 1.3, 0.5, and 0.5% for hydrostatic pressure alone, pressurized with pediocin AcH, pressurized with BP_X, and pressurized with BP_Y, respectively. Rancidity and discoloration were detected in roast

beef samples both in uninoculated control (control negative) and treated products after 3 weeks of storage at 25°C. Discoloration and rancidity were also detected in both uninoculated control and treated summer sausage after 3 and 5 weeks storage at 25°C, respectively. The color of roast beef changed from pinkish brown to brown. The color of summer sausage changed from bright pinkish brown to pale pinkish brown. Both rancidity and discoloration of processed meat products are due to oxidation from the oxygen remaining in the bag. During prolonged storage, organic peroxide is formed(2).

CONCLUSIONS

1. Pressurization at 50,000 psi for 5 min at 50°C caused some viability loss to spoilage and pathogenic bacteria inoculated on roast beef, cotto salami or summer sausages.
2. Pressurization in the presence of bacteriocin-based biopreservatives increased more viability loss to spoilage and pathogenic bacteria inoculated on processed meat products. Biopreservatives may contribute to the residual bactericidal effect during storage.
3. Under the study conditions, microbial shelf-life of processed meat products increased to 12 weeks at 25°C.
4. pH of the product played an important role in greater reduction of pathogenic bacteria reflecting safety enhancement in pressurized processed meat products stored at 25°C.

REFERENCES

1. Banwart, G.J. 1979. Conditions that influence microbial growth in food. p. 116-185, *In* Basic Food Microbiology. AVI Publishing Co., Inc., Westport, CT.

2. Bard, J. and Townsend, W.E. 1978. Factors influencing quality of cure meats. *In* Price, J.F. and Schweigert, B.S. eds., The Science of Meat and Meat Products, 2nd ed., p. 452-483. Food & Nutrition Press, Westport, CT.

3. Biswas, S.R., Ray, P., Johnson, M.C. and Ray, B., 1991. Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. Appl. Environ. Microbiol. 57:1265-1267.

4. Carlez, A., Cheftel, J.C., Rosec, J.P. and Richard, N. 1992. Effects of high pressure and bacteriostatic agents on the destruction of *Citrobacter freundii* in minced beef muscle. Collq. INSERM 224:365-368.

5. CDC 1981a. Annual summary, 1978. Foodborne disease surveillance. Center for Disease Control, Atlanta, GA.

6. CDC 1981b. Annual summary, 1979. Foodborne disease surveillance. Center for Disease Control, Atlanta, GA.

7. CDC 1983a. Annual summary, 1980. Foodborne disease surveillance. Center for Disease Control, Atlanta, GA.

8. CDC 1983b. Annual summary, 1981. Foodborne disease surveillance. Center for Disease Control, Atlanta, GA.

9. Cheftel, J.C. 1992. Effects of high hydrostatic pressure on food constituents: an overview. Colloq. INSERM 224:195-209.

10. Cheftel, J.C. and Culioli, J. 1996. Effects of high pressure on meat: a review. 42nd IcoMST Manuscript Lillehammer, Norway, 1-6 September 1996.

11. El Moueffak, A., Cruz, C., Antoine, M., Montury, M., Demazeau, G., Largeteau, A., Roy, B. and Zuber, F. 1995. High pressure and pasteurization effect on duck foie gras. Internl. J. Food Sci. Technol. 30:737-743.

12. Farr, D. 1990. High pressure technology in food industry. Trends Food Sci. Technol. 1:14-16.

13. Genigeorgis, C. 1986. Problems associated with perishable processed meats. Food Technol. 40:140-154.

14. Hauben, J.A.K., Wuytack, E.Y., Soontjens, C.F.C. and Michiels, C.W. 1996. High-pressure transient sensitization of *Escherichia coli* to lysozyme and nisin by disruption of outer-membrane permeability. J. Food Prot. 59:350-355.

15. Hood, T. 1984. The chemistry of vacuum and gas packaging of meat. p. 213-230. *In* Recent Advances in The Chemistry of Meat. Allen J. Bailey (ed.), The Royal Society of Chemistry, Burlington House, London.

16. Kalchayanand, N., Hanlin, M.B. and Ray, B. 1992. Sublethal injury makes gram-negative and resistant gram-positive bacteria sensitive to the bacteriocins, pediocin AcH and nisin. Lett. Appl. Microbiol. 15:239-243.

17. Kalchayanand, N., Sikes, A., Dunne, C.P. and Ray, B. 1994. Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. Appl. Environ. Microbiol. 60:4174-4177.

18. Lechowich, R.V. 1978. Microbiology of meat. *In* Price, J.F. and Schweigert, B.S. eds., The Science of Meat and Meat Products, 2nd ed., p. 230-286. Food & Nutrition Press, Westport, CT.

19. Mackey, B.M., Forstiere, K. and Issaca, N. 1995. Factors affecting resistance of *Listeria monocytogenes* to high hydrostatic pressure. Food Biotechnol. 9:1-11.

20. O'Brien, J.K. and Marshall, R.T. 1996. Microbiological quality of raw ground chicken processed at high isostatic pressure. J. Food Prot. 59:146-150.

21. Patterson, M.F., Quinn, M., Simpson, R. and Gilmore, A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and food. J. Food Prot. 58:524-529.

22. Pearson, A.M. and Tauber, F.W. 1984. Processed Meats, 2nd ed. AVI Pub. Co., Inc. Westport, CT.

23. Petran, R.L. and Zottola, E.A. 1989. A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. J. Food Sci. 54:458-460.

24. Ray, B. 1992. Pediocin(s) of *Pediococcus acidilactici* as a biopreservative. p. 265-322. *In* B. Ray and M.A. Daeschel (eds.), Food Biopreservatives of Microbial Origin. CRC Press, Inc., Boca Raton, FL.

25. Ray, B. 1993. Sublethal injury, bacteriocins, and food microbiology. ASM News 59:285-291.

26. Shigehisa, T., Ohmori, T., Saito, A., Taji, S. and Hyashi, R. 1991. Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. Internl. J. Food Microbiol. 12:207-216.

27. Schillinger, U., Geisen, R. and Holzapfel, W.H. 1996. Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. Trends Food Sci. Technol. 5:158-164.

28. Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. 1986. Bergey's Manual of Systematic Bacteriology. Vol. 2. Williams & Wilkins, Baltimore, MD.

29. Stewart, C.M., Jewett, F.F., Dunne, C.P. and Hoover, D.G. 1997. Effect of concurrent high hydrostatic pressure, acidity and heat on the injury and destruction of *Listeria monocytogenes*. J. Food Safety 17:23-26.

30. USDA. 1983. Statistical summary. Federal meat and poultry inspection for fiscal year 1982. FSIS, U.S. Dept. of Agriculture, Washington, DC.

31. Vedamuthu, E.R., Raccach, M., Glatz, B.A., Seitz, E.W. and Reddy, M.S. 1992. Acid-producing microorganisms. p.225-238, *In* Carl Vanderzant and Don F. Splittstoesser (eds.), Compendium of Methods for The Microbiological Examination of Foods 3rd ed. American Public Health Association, Washington, DC.

32. Yang, R., Johnson, M.C. and Ray, B. 1992. Novel method to extract large amounts of bacteriocins from lactic acid bacteria. Appl. Environ. Microbiol. 58:3355-3359.